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Previews

NOTCHing an Arrow at Cord Blood: Translating Stem Cell Knowledge into Clinical Practice

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Umbilical cord transplants have emerged as an important therapeutic option for patients with leukemia and lymphoma. A recent study in *Nature Medicine* highlights the potential and challenges of translating cell-based therapies to the clinic.

As the world awaits the much-anticipated translational application of the recent advances in embryonic and induced pluripotent stem cell research, human stem cell therapy has been a quiet reality for over half a century for patients in need of replacement of their blood-producing system. Hematopoietic stem cell (HSC) transplantation has been increasingly refined since the groundbreaking work of Donnall Thomas (Thomas et al., 1957) in the late 1950s, and currently, more than 3600 patients undergo transplantation each year in the U.S. HSC transplants often offer the only chance for cure for patients with leukemia, lymphoma, and other blood disorders; while increasingly successful, risks remain due to the conditioning regimen, prolonged absence of immunocompetent cells, and graft-versus-host disease. Bone-marrow-derived HSCs, as well as HSCs harvested from the peripheral blood stream following cytokine-induced mobilization, have proven to be widely effective therapeutic agents.

Umbilical cord blood (UCB), obtained from the placental and umbilical vessels after birth, has emerged as another important source of HSCs over the last two decades (Broxmeyer et al., 2009), with more than 20,000 patients treated worldwide to date. Currently, UCB is the HSC source for almost 20% of all transplants in the U.S. and for 50% in Japan. UCB is a particularly important resource when no matched HSC donor can be found in the registry, as is often the case for patients from ethnic minorities. UCB HSCs have the dual advantage of being readily available when needed and relatively immature, requiring less stringent immunohistocompatibility matching than adult-derived HSCs while providing an overall more efficient immunoreconstitution. However, due to the physical volume of the cord itself, the number of available HSCs per UCB unit is approximately 10% of that commonly utilized in conventional transplants. This results in delayed HSC engraftment and recovery of white blood cell number (~28 days for UCB versus 18–20 days for conventional transplants), leaving the patient at increased risk for infections. One attempt to enhance HSC engraftment has been to routinely transplant two UCB specimens (Ballen et al., 2007); while this approach boosts total HSC number infused, the time for neutrophil recovery has not been significantly affected.

To date, no treatment strategy has been shown to enhance engraftment and neutrophil recovery. A recent study published in *Nature Medicine* (Delaney et al., 2010) by Delaney et al. provides a proof-of-concept to alleviate this unmet clinical need. This work represents the culmination of a translational effort that began with the discovery of the *NOTCH1* gene in human $CD34^+$ stem/progenitor cells 16 years ago (Milner et al., 1994). Here, Delaney et al. used an ex vivo culture strategy to significantly expand UCB stem and progenitor cell types over 3 weeks through activation of the Notch pathway by synthetic Delta ligand. The authors first demonstrate the translational potential of their earlier work, presenting the results of xenograft transplants into immunodeficient SCID mice, where ex vivo culture with Delta increased the kinetics and efficacy of bone marrow engraftment. By using a technically challenging limiting dilution xenotransplantation scheme, they calculated a 6.2-fold enrichment in SCID-repopulating cells following Delta exposure. Together, these data clearly demonstrated that human UCB cells were responsive to Notch activation through in vitro culture.

Delaney et al. then present the preliminary results of the first ten patients enrolled in a phase I clinical trial using the Delta in vitro culture protocol for UCB transplantation. Here, the most impressive finding is the significantly decreased average time of neutropenia (defined by an absolute neutrophil count of <500 cells per μl) from 26 days to 16 days when compared to patients receiving a standard regimen, with the majority of the myeloid recovery derived from the Delta-treated UCB unit. Prolonged neutropenia has been correlated with increased rates of both bacterial and fungal infections, representing one of the major complications after HSC transplantation. These data highlight the potential of the presented culture method to significantly improve clinical practice. While not presented in detail here, once tested in a larger cohort with long-term follow-up, the dramatically improved neutropenia interval should have clinically measurable consequences, such as decreased hospital stays, infectious complications, and possibly long-term survival.

The current study also highlights the challenges that exist when translating basic stem cell biology into clinical practice, beginning with the selection of the most appropriate culture containers and

choice of media and cytokine additives, which are only hinted at in the manuscript. These seemingly trivial issues could significantly impair the results of clinical trials and potentially prevent novel treatment strategies from demonstrating improvements. Additionally, there are different measures of effectiveness between preclinical and clinical studies: the ability of HSCs to engraft marrow in a long-term repopulation assay has long been the gold standard in murine HSC work and in fact is the functional definition of HSCs. However, in clinical practice, engraftment percentage, while elegantly documenting functional efficacy, does not directly translate into improved patient care. Here, duration of neutropenia and subsequent length of hospitalization are much more relevant endpoints and are rarely assessed in murine assays. One of the biggest challenges that may be faced by the Delta-mediated expansion approach is the effect of the culture conditions on the long-term viability and function of HSCs: only 1 of 10 patients had evidence of fully functional engraftment of the Delta-cultured UCB unit, while one other maintained only the myeloid population, indicating impaired long-term HSC function in the vast majority of treated UCB specimens. The authors themselves note this issue and speculate that the culture altered the self-renewal potential of the HSCs. It is currently poorly understood how the culture conditions—here, a mix of five cytokines—or the isolation of the starting population, such as $CD34^+$ UCB cells, alters the differentiation capacity and self-renewal potential of the HSCs; however, the maintenance of stem cells of any tissue source in culture, including embryonic stem cell derived, is a uniformly challenging problem. The culture conditions that help expand the cell population of interest may effectively deprive the HSCs of microenvironmental or niche components that are required for optimal HSC persistence. Delaney et al. use the biologically relevant Notch pathway to enhance the proliferation of phenotypic UCB HSCs; in this issue of *Cell Stem Cell* (Butler et al., 2010), members of the same group show that one possible physiological source of Notch in the bone marrow niche may be endothelial cells (ECs). UCB units, harvested directly from their endothelial niche, contain a mix of immature HSCs and ECs; perhaps the presence of these ECs contributes to the overall more efficient immunoreconstitution seen with UCB transplants. One can speculate whether the addition of another factor as the physiological "balancer" of Notch signaling, possibly also EC derived, would be able to help maintain these cells in a functional HSC state during expansion.

In principle, three different approaches to enhance engraftment and count recovery after transplantation are possible: ex vivo culture to enhance HSC and/or progenitor number, as done here or with the use of insulin-like growth factor (Zhang et al., 2008); short-term ex vivo treatment of UCB prior to infusion, as in recent studies using prostaglandin E2 (North et al., 2007) or an inhibitor of CD26 (dipeptidylpeptidase IV) (Campbell et al., 2007); or treatment of the recipient to enhance the function of the hematopoietic niche, such as parathyroid hormone (Adams et al., 2007). Successful treatment strategies may be combined to maximize the benefit for the patients. But "more" will not always be "better": treatment of the transplant recipient with leukemia or lymphoma may increase the risk of stimulating cancer cells with the transplanted HSCs, and enhanced differentiation of progenitor cells to improve short-term engraftment and cell recovery, as shown by Delaney et al., may impair the stem cell pool. Future studies are needed to address these and other questions to improve safety and efficacy of UCB transplants; Delaney et al. (Delaney et al., 2010) have demonstrated that clinical translation to enhance stem cell-based therapies can be accomplished and indicate the challenges for the field on the road ahead.

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